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VARIATION AND LIFE CYCLES OF PATHOGENIC BACTERIA

ONE PLATE

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In his "Species Plantarum," Linné points out the great variability of fungi. He found it difficult to recognize and limit the species. For the pathogenic bacteria this difficulty continued a much longer time. Nägeli denied the existence of constant species and believed that the same species produced lactic acid and caused scarlet and typhoid fevers and other diseases. In his laboratory the cultures of B. subtilis were changed to B. anthracis. R. Koch[1] deserves great credit for proving the constancy of many pathogenic bacteria. In the earliest studies he discovered the endogenous spores of B. anthracis and mentioned several extraordinary forms. He evidently soon lost interest in such forms, however, and now their existence is denied by most bacteriologists or they are considered degenerative products or involution forms. Thus science has gone from one extreme to the other; scientists have striven to make Nature less complicated than it is.

Löhnis,[2] in an important work in 1921, cites a great number of authors, some earlier than Koch, who have studied the atypical forms of bacteria. Many are easily recognized from plates and descriptions. The descriptions of variation and life cycle of a species are, however, difficult to interpret. For such observations the pure culture is necessary.

In 1881, I studied B. typhosus in patients and found in the living blood both motile and immotile rods and in the dead human body the oval rods of Eberth-Koch. The following year, I had to combat a great epidemic of typhoid fever. The observations made gave me ideas for a study of B. typhosus, which I still continue. I then came to the conclusion that neither the pathogenicity of this organism nor the epidemiology of typhoid fever can be fully explained until the life cycle of B. typhosus has been thoroughly investigated.

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[1] Zur Untersuchung von pathogenen Organismen, Mittl. d. Keis. Gesundh. Amts., 1881, 1, p. 1.

[2] Studies upon the Life Cycles of the Bacteria, Memoirs National Acad. Sciences, 1921, 16, p. 335.

LIFE CYCLES OF SOME PATHOGENIC BACTERIA

In the first two decades of my studies I occasionally found new forms of great interest, for example, plasmodia and microconidia of B. typhosus; but I was not able to produce them ad libitum. Now I conclude that low temperature had effected these results. Since 1908 I have used, to a great extent, low temperature and drying mediums for my cultures. Thus it is easy to produce the new varieties in great abundance. Burri's method of one-cell culture has further advanced my studies. In my laboratory Troili-Peterson[3] successfully modified Burri's method.

It is quite necessary to discern common variation from the constant variety that is never able to go back to the original. Linné speaks both of variation under the influence of environment and of constant varieties. He found the latter numerous among the higher plants, and he was not able to reproduce the original form by culture. Linné's principle is obvious, but in order to prove the constancy of a bacterial form, one must work for years. Nobody can pretend to know the complete life cycle and all the varieties of a bacterial species. It would be an assumption to think so, at least in our day.

The important varieties must be sharply distinguished from all degenerated or dying forms. Thus each variety must be examined to determine its origin and its development. The literature of bacteriology is abundant in descriptions of extraordinary bacterial forms without concise statements; frequently it was not even determined whether the forms were living or dead.

B. typhosus.—On drying substrate at 10-14 C. the common rods become transformed in a remarkable manner; they grow larger and often produce exogenous globules terminally and laterally; the membranes seem to dissolve; the protoplasm protrudes; the rods lose their form and produce protoplasmic masses, plasmodia. The exogenous conidia germinate immediately to new rods and globules; sometimes I saw them motile (figs. b, d and e).

We often find small immotile free granules in great numbers. They are probably produced when the substrate is poor, dry and cold. Figure c presents beautiful lateral microconidia, a rare occurrence.

All is rapidly changed when the transformed culture is transferred to a good substrate at 37 C. The rods sprout and produce large conidia that germinate immediately. The large globules, acting as sporangia, open; globules and fine curved chains appear. The contents look as shown in f; the cell wall is often visible at the side. The whole phenomenon occurs within a few hours. The next day we see only common rods.[4]

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[3] Ein-Zell-Kultur von langsam wachsenden Bakterienarten, etc., Centralbl. f. Bakteriol., ii, 1914, 42, p. 526.

At low temperature, e. g., in broth, the myceloid prevails. We find long, thick, intricate threads that often produce large conidia. The long, thin threads formed in poor nutritive solution are inclined to bud to microconidia.

In all cultures we find needle-shaped rods, fine and motile, 2 microns long. On poor substrates the rods are often very frequent and keep their constancy fairly well. I think they are of the same origin as the microconidia.

Eberth and Koch saw in the dead human body chiefly aggregates of large oval rods, 2 microns long, which took the stain weakly. This variety is remarkable, though almost forgotten. Perhaps it constitutes a middle form between plasmodium and common rod.[5]

All examined varieties of B. dysenteriae agree with those of B. typhosus. The life cycles are similar but not identical. Spirillum cholerae agrees also strikingly with B. typhosus. At 14-15 C. on drying substrates the spirilla develop quickly into thick and long forms and reproduce through budding conidia which promptly germinate. At the same time small amorphous plasmodia appear (figs. i and 1). After a month the threads look empty, and the culture is replete with germinating conidia. The conidia are often motile; however, they are immotile at low temperature and are then more easily observed. It is interesting to follow the growth of the conidia to spirilla; usually one spirillum breaks forth, sometimes two from the same globule. The next day only small spirilla are to be found in the culture.[4]

In the year 1852, Perty[6] discovered both endogenous spores of a bacillus and exogenous conidia or sporangia of Spirillum undula. He saw the spirilla move with lateral and terminal globes, having a diameter of 2 microns and quite distinct outlines. Nevertheless, Perty declared that the exogenous bodies were coagulated clumps.[7] Beyond doubt they were able to sprout.

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[4] Almquist, E.: Studien über das Verhalten einiger pathogenen Mikroorganismen, Centralbl. f. Bakteriol., I. O., 1908, 48, p. 175. Wuchsformen, Fruktifikation und Variation der Typhusbakterie, Ztschr. f. Hyg. u. Infektionskr., 1916, 83, p. 1.

[5] Footnote 1, p. 45. Gaffky: Zur Aetiologie des Abdominaltyphus, Mitteilungen d. Kais. Gesundh. Amts., 1884, 2, p. 372.

[6] Zur Kenntnis kleinster Lebensformen, 1852.

[7] Ibid., p. 132.

In laboratory cultures of Sp. cholera we find many large globules after a short time. So far as I know, no one has studied their origin or development. Kitasato [8] apparently believes that they are dead. I believe so also. Ferran and Hüppe describe forms of Sp. cholera similar to arthrospores but without resemblance to the exogenous germinating conidia. Gamaleia [9] used substrates containing lithium and saw many globules and other forms replete with crystals of lithium. He found it difficult to ascertain whether these globules were living.[10]

Olsson [11] made interesting studies of the life cycle of the same spirillum. In certain mediums they were long and immotile; the colonies were not smooth but wrinkled. Gradually all the organisms assume this changed form permanently. The transformation is most rapid at about 20 C.; it can be completed within half a year. It is surprising that these immotile spirilla return to the normal form when grown for one month on agar at 37 or at 17 C. Olsson found that the virulence was then increased.[11]

B. diphtheriae.—I have used long, granular, virulent rods from single cell cultures (fig. N). On a lactate substrate the rods transform themselves within 2 or 3 weeks at 19 or 26 C. into fine chains, similar to oidia (fig. o, p). They live on the same substrate several months as a kind of resting form and are easily observed after having been stained. When the oidia are transferred to serum at 37 C. they grow immediately to typical rods. After one day all are changed to rods.

In drying broth the rods are transformed into large masses within a month at 35 and at 17 C. In addition to clumps we see also thick rods with branches and exogenous buds. Such cultures rapidly produce fine forms on serum at 37 C. (fig. r). Within a few days only normal rods are visible (*N*).

The virulence increases by passing through the described cycle. Koraen and I [12] were also able to make a culture of very fine needles 1-2 microns in length which was quite virulent. In drying broth the latter strain turned partially into large masses within 7 weeks at 22 and also at 18 C.[6]

Many authors have described other forms of B. diphtheriae. Only by studying single-cell cultures, will their importance be revealed.

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[8] Die Widerstandsfähigkeit der Cholerabakterien gegen Eintrocknen, Ztschr. f. Hyg. u. Infektionskr., 1889, 5, p. 34, and 6, p. 11.

[9] Elemente der allgemeinen Bakteriologie, 1900.

[10] Ibid., p. 215.

[11] Zur Variation des Choleravirus, Centralbl. f. Bakteriol., I., O., 1915, 76, p. 23.

[12] Studien über Biologie und Wuchsformen der Diphtheriebakterie, Ztschr. f. Hyg. u. Infektionskr., 1918, 85, p. 347.

Micrococcus thulini.—This coccus appeared in Stockholm during the Spanish fever in 1917 and grew in chronic wounds and in the throat as a streptococcus. On agar the same form turned into a large staphylococcus-like form (fig. S). After a short time the cultures began again to reproduce small streptococci and soon lost their vitality (fig. t). In broth at 35 C., the small cocci are always more numerous (fig. u).

On drying agar we find very small buds on the micrococci; M. pyogenes-aureus will act in the same way under such circumstances. The buds constitute a kind of microconidium and develop rapidly into cocci on good substrates. After passing the cycle, the small cocci reproduce the large cocci (fig. S). From Koraen's [13] observations we conclude that M. thulini gains new energy in passing the cycle.

BACTERIAL MUTATION

The study of this important question began when Jenner discovered cowpox. Today, after a great deal of experience, we know that the virus of cowpox does not cause smallpox. This virus has been transformed in the body of the cow and causes another disease with shorter incubation. Thus a constant variety, a real mutation, has appeared.

B. typhosus.—In the year 1910 I began to separate with the Berkefeld filter the small granules that I often encountered in my saprophytic cultures. From 100 experiments I 10 times obtained cultures of filtrable bacteria that did not go back to the origin, B. typhosus. These strains produce in the rabbit a serum that agglutinates B. typhosus in a dilution of 1 : 500 and also gives good effect in Pfeiffer's test. I have called them B. antityphosum.[14]

I have cultivated these strains for 11 years and have never seen them revert; they are beyond doubt constant. At low temperature and on poor substrate they grow more readily than B. typhosus; therefore I thought it might be possible to separate the granules from the rods in pure cultures of B. typhosus. Sometimes I succeeded in isolating the granules by culture.

A third method has also occasionally given positive results, i. e., new vigorous constant forms were obtained. After heating at 60 C. the appearance is much changed, the plasmodia prevail, and the rods assume monstrous shapes. They agglutinate somewhat like B. typhosus.

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[13] Studien über Umformung von Mikrokokken in trocknender Kultur, Ztschr. f. Hyg. u. Infekionskr., 1918, 85, p. 359.

[14] Studien über filtrierbare Formen in Typhuskulturen, Centralbl. f. Bakteriol., I, O., 1911, 60, p. 167.

Different strains are gained by filtering, culturing and heating; but the same method gives different forms. I obtained different granules by filtering and culturing. The granules are different also in the cultures of B. typhosus. In the plate the single microconidia are not similar to the curved chains in figures f and g. In cultures I obtained strains more similar to the free granules. On the other hand, B. antityphosum appears both as curved chains and as free granules, sometimes forming a short chain. To be sure, B. typhosus produces by mutation several different varieties.

Spirillum cholera.—By heating at 60 C. I have received some new varieties. One of them I have studied for about 8 years. It appears in different forms, as a little motile needle organism, as a globule or plasmodium, and agglutinates by cholera serum.

In the bacteriologic literature we find much about "mutation;" most authors, however, do not mean new constant varieties, but only a sudden change, e. g., the unusual appearance of the colonies, the faculty to ferment a new kind of sugar, etc. In my opinion the observations on the asporogenous B. anthracis have more interest with regard to mutation. As far as I know, no one has continued these experiments long enough. E. C. Hansen studied asporogenous saccharomyces varieties for sixteen years and proved their constancy.

In 1910, Herzog[15] suggested that the bacteria of trachoma descend from Micrococcus gonorrhea. A large coccus was said to be transformed into a very small one, and the new form was found to be able to live and multiply as such.[16] It will be interesting to learn whether the small cocci are able to reproduce the larger forms.

Beyond doubt several pathogenic strains originate from other bacterial species. Primarily I think of the coccus of pemphigus and impetigo contagiosa, M. pemphigi-neonatorum.[17] We find many cocci in the vesicles. They are easy to isolate by plating and agree almost completely with M. pyogenes-aureus. The difference is pathologic; one coccus produces limpid vesicles in the skin, the other pus. I discovered this in 1890, and for almost thirty years we have continued these observations.[13] In the United States the same results have been obtained by Clegg and Wherry[18] and by Falls.[19]

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[15] Ueber die Natur and Herkunft des Trachomerregers, 1910.

[16] Ibid., p. 46.

[17] Almquist, E.: Pemphigus neonatorum, etc., Ztschr. f. Hyg. u. Infektionskr., 1891, 10, u. 253.

[18] The Etiology of Pemphigus Contagiosus, Jour. Infect. Dis., 1906, 3, p. 165.

[19] The Bacteriology of Pemphigus Neonatorum, ibid., 1917, 20, p. 86.

It is remarkable that granules from a bacterial species can be separated from the culture by filtering and are able to multiply as such. Some years ago Löhnis partially verified these observations by filtering saprophytic bacteria. He writes: "Gonidia grow to new cells very readily while still connected with the parent cell, or if they have otherwise access to genuine plasma, like that in dead or dying cells of fungi and algae. But in other cases free gonidia have rarely been found to be inclined to act promptly as normal reproductive organs when kept under ordinary laboratory conditions." [20] The isolated granules do not grow readily. By making use of substrates containing lactose, lactate, sterile algae or sterile fecal matter I succeeded. Now after eleven years the same strains are adapted also to agar and to a temperature of 37 C.

The second difficulty is to verify the granules. The agglutination and Pfeiffer's test were decisive for numerous strains. The similarity to the granules in the cultures of B. typhosus helps to confirm their identity, but errors may occur. Thus far I have published only my results about agglutinating strains, produced by heating, and B. antityphosum by filtering.[14]

It is remarkable that it is possible to separate a minute part of B. typhosus, which is able to multiply as such. Beyond doubt we stand here at the beginning of an analysis of a bacterium, that is to say, of a living organism in quite a new manner.

B. antityphosum also passes through a life cycle. It appears usually as minute ovals, grows larger on sugar substrates, produces a small branched plasmodium as a promycelium, thrives well at 10 C. and competes there successfully with B. typhosus; on certain substrates it grows in thick layers. In comparison with the long, motile parent cells it is much reduced. We must expect to find similar minute forms in our environment. Probably most of them are difficult to cultivate.

It is obvious that these minute forms must be necessary for the parent strains, though they also may have their own existence. We believe that they readily fuse with other forms of the same strain and cause a kind of synthesis and it seems not impossible that a synthesis takes place also with foreign forms. In the symplastic stage we must expect surprises of various kinds. The bacterial synthesis and analysis are of great importance to biology.

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[20] Footnote 2, p. 183.

IMPORTANCE OF VARIATION

The variation of the fungi is manifold; even the same species may produce many different spores. The bacteria perhaps vary still more. We already know true series and different generative bodies of many species.

It must be borne in mind that all variation helps the species itself. In old cultures we often find a periodicity of growth; after an interval secondary colonies appear, and after another interval perhaps tertiary colonies, according to the ability of the strain to adapt itself to new conditions. For variation and adaptation the plasmodium and the whole life cycle are essential. Löhnis suggests that the bacteria in the symplastic stage are in a much better position to adapt themselves to changed environmental conditions than they are during their cellular life.[21]

Perty has referred to the plasmodium as a very fine "Punktsubstanz." He saw the cells dissolve and form the plasmodium, which was transformed into new organisms of the lowest kind.[22]

For the pathogenic bacteria a low temperature and a drying substrate are of great importance in producing plasmodia and regenerative bodies. A kind of rejuvenation takes place often after passing the life cycle. The new forms often exhibit great constancy, for instance, in the loss of motility, in their power to produce conidia, etc.

Increased virulence is another rather common occurrence when pathogenic bacteria are grown saprophytically. Koraen found first that some strains of B. typhosus when grown at low temperature gain resistance against agglutinating serum and the lytic power of the blood. We also found that the virulence can be increased when B. typhosus is cultivated for a long time. Olsson discovered that Spirillum cholerae became more virulent after having passed a long series of development.[11] B. diphtheriae is able to recover its virulence after it has passed the plasmodium stage. The same was observed with an avirulent degenerated strain.

The common method for augmenting the virulence is the "passages" from animal to animal, but this method cannot claim general validity. In some epidemics we observe that the "passages" from man to man diminish the virulence and at last extinguish it completely, e. g., in measles. In the saprophytic growth many bacteria regain their pathogenicity; probably this is quite common with many species.

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[21] Footnote 2, p. 196.

[22] Footnote 6, p. 113.

Pathogenicity.—The textbooks often know more than the research workers. We read that the common rods of B. typhosus pass through the intestines, rest in the glands, etc. However, who has studied the forms of B. typhosus in the living human body? The big oval forms in the dead human body are well known, but still their origin is in doubt. Perhaps they constitute an intermediate stage between plasmodium and rod.

In the living blood of typhoid patients I observed, in 1881, in Ranvier's moist chamber before coagulation took place: (1) common rods with their characteristic motility—rare, but sometimes in abundance; (2) a little immotile needle, fairly common; (3) rather big oval forms, rare. All three forms took the stain easily. After a few days I discovered in my hanging drops occasional granules germinating to a kind of fine plasmodium, similar to the plasmodium of B. antityphosum.[23] The phenomenon was not common, but in one case I found 20 germinating granules in the same drop. These observations should be continued.

Within a week the common rods of B. typhosus, B. dysenteriae and Spirillum cholerae may be transformed by the saprophytic growth. They are then able to produce plasmodia and conidia. In the human body each rod of B. typhosus and B. dysenteriae may produce a number of the smallest forms which easily pass the intestinal wall. The minute size and their great number make the passage easier for them than for the rods. Perhaps the plasmodium and the granules constitute the chief forms within the sick body.

Epidemiology.—A chiefly mechanical theory is insufficient to explain epidemics. Season, meteorology, etc., are important factors in the appearance of many epidemics, but most authors pay little attention to the relation between seasons and epidemics, and to the causes of the difference in the virulence of the strains.

The study of the different stages in the development of the bacteria will make it possible to explain both the pathogenicity and the rise and decline of many epidemics. Plasmodia and fructification facilitate immensely the invasion of new individuals. Furthermore, the saprophytic growth restores the virulence, e. g., of B. diphtheriae. It is well known that new cases of diphtheria in the same family often occur after one or two months. This fact may be explained by the increased virulence, since the bacteria have passed the stage of plasmodium. On the other hand, minute, avirulent forms, similar to B. antityphosum, possibly help to make a population immune.

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[23] Footnote 4, second part, plates 14 and 15.

Classification.—In 1899 Willibald Winkler[24] of Vienna pointed out the great importance of the plasmodia that are able to produce bacteria, "Teilungsplasmodia" and sporangia. Probably all colonies and the so-called zooglea develop from plasmodia. The branched forms of B. tuberculosis, B. leprae, B. diphtheriae, etc., are connected with the plasmodial stage. The bacteria are declared to be related to the myxomycetes. These important discoveries and theories will probably sometime be regarded as the starting point for a natural system of bacteria. Thus far the authors have been too much occupied with subjective essays of ephemeral nature on bacterial classification.

TERMINOLOGY

For creating standard terms our knowledge is too incomplete. We must wait until both the bacteria and the other classes of fungi have been investigated more thoroughly. At present the chief endeavor is to observe and describe exactly, and to use appropriate terms.

In my earlier papers I pointed out the temporary value of my own terms. I looked for analogies in the mycology of DeBary and of Bredfeld and added bacterial to each term. Thus I speak of bacterial plasmodia, bacterial conidia, etc., because I cannot decide whether my findings agree with those concerning plasmodia, etc., in other parts of the mycology.

Bacterial mutation.—Formation of a new constant variety or species. Except hybrids, probably all new species are produced by mutation.

Bacterial plasmodium.—Protoplasmatic clump, the amorphous stage of the species (Figs. e and q). Löhnis calls it symplasm. It is somewhat similar to the promycelium of Tulasne.

Conjunction consists of the direct union of two or more individual cells.[25] Perty has spoken of this; copulation is a similar phenomenon—a creation of new individuals.[26]

Myceloid.—Long, intricate threads without branches.

Bacterial conidium.—Exogenous globule sprouting to a rod, spirillum or a new globule (Figs. b to f and i to m); Löhnis calls it regenerative body or exospore.

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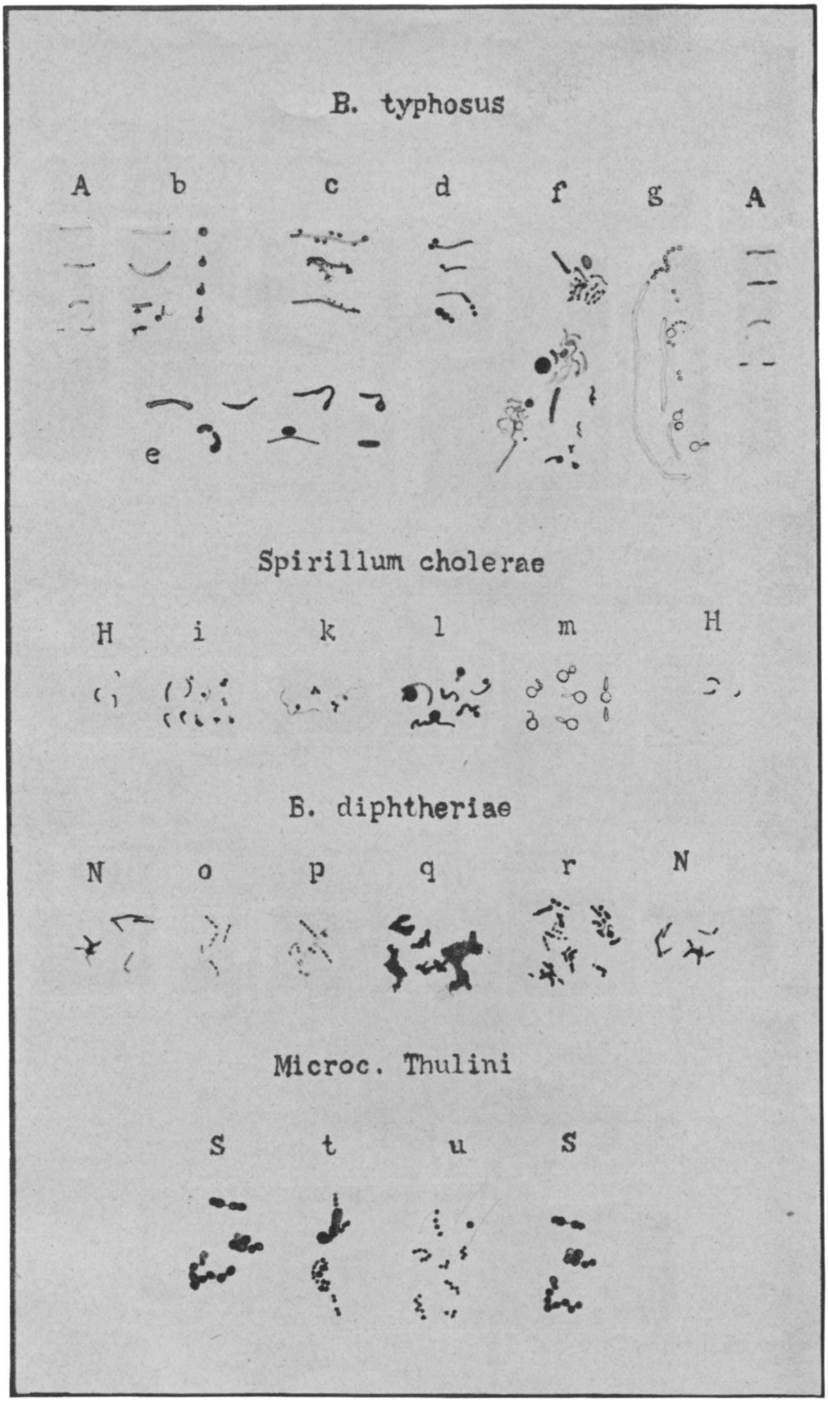
[24] Untersuchungen über das Wesen der Bakterien, Centralbl. f. Bakteriol., ii, 1899, 5, p. 569.

[25] Footnote 2, p. 197.

[26] Footnote 6, p. 220.

PLATE I



The figures are drawn from photographs; only figs. *g* and *m* were made directly from the microscopic observation; × 1,000. B. typhosus: A, rods of different size; b to e, their development on drying substrate at 10-14 C., b on agar for one week; thick rods with lateral and terminal conidia, free conidia germinating; c, lateral microconidia; d, after 4 weeks on agar; e, after 3 weeks on potato, plasmodia; f, transformed cultures germinating on serum at 37 C.; three sporangia are open, their content is made up of finest curved chains and globules; g, germination directly observed under the microscope. Spirillum cholerae: H, common spirilla; i, the same after a few days at 15 C.; longer and larger forms. plasmodia, conidia, some germinating; k, on drying agar at 14 C. after one month; empty threads, conidia; l, transformed culture 4 hours at 37 C; thread and germinating conidia; m, the same culture 4 hours at 22 C.; conidia germinating to spirilla and globules. B. diphtheriae: N, Löffler's rod; o, p, on lactate substrate 2 to 3 weeks at 19 or 25 C., similar to small oida; q, in drying broth at 37 C. after one month; big clumps—plasmodia; r, the clumps turning to rods. Micrococcus Thulini: S, great cocci similar to staphylococci; t, the great cocci after 5 days begin to produce small cocci, similar to streptococi; u, in broth at 35 C. after 2 weeks; fine cocci only, on agar large cocci are reproduced.

Bacterial microconidium.—A very small exogenous granule with a diameter of about ½ micron (Fig. c). Löhnis uses the term gonidium.

Bacterial sporangium.—Large exogenous conidium, containing several cells, globules or chains (Figs. f and g). Löhnis prefers the term gonidangium.

Bacterial oidium is produced by the segmentation of a rod or thread (Figs. o and p). In 1890, I introduced the term oidium for the spores of Streptothrix or Actinomyces.[27] The type is Oidium lactis. This spore is also named arthrospore.

Bacterial micro-oidium is a very small oidium, difficult to observe with the microscope, better visible in the photographs (Figs. f and g).

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[27] Untersuchungen über einige Bacteriengattungen mit Mycelien, Ztschr. f. Hyg. u. Infektionskr., 1890, 8, p. 189.